



Temperature of embryo culture for assisted reproduction

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Temperature of embryo culture for assisted reproduction

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What's new

Date	Event	Description
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History

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Abstract

Background

'Infertility' is defined as the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse. One in six couples experience a delay in becoming pregnant. In vitro fertilisation (IVF) is one of the assisted reproductive techniques used to enable couples to achieve a live birth. One of the processes involved in IVF is embryo culture in an incubator, where a stable environment is created and maintained. The incubators are set at approximately 37°C, which is based on the human core body temperature, although several studies have shown that this temperature may in fact be lower in the female reproductive tract and that this could be beneficial. In this review we have included randomised controlled trials which compared different temperatures of embryo culture.

Objectives

To assess different temperatures of embryo culture for human assisted reproduction, which may lead to higher live birth rates.

Search methods

We searched the following databases and trial registers: the Cochrane Gynaecology and Fertility (CGF) Group Specialised Register of Controlled Trials, the Cochrane Central Register of Studies Online, MEDLINE, Embase, PsycINFO, CINAHL, clinicaltrials.gov, The World Health Organization International Trials Registry Platform search portal, DARE, Web of Knowledge, OpenGrey, LILACS database, PubMed and Google Scholar. Furthermore, we

manually searched the references of relevant articles and contacted experts in the field to obtain additional data. We did not restrict the search by language or publication status. We performed the last search on 6 March 2019.

Selection criteria

Two review authors independently screened the titles and abstracts of articles retrieved by the search. Full texts of potentially eligible randomised controlled trials (RCTs) were obtained and screened. We included all RCTs which compared different temperatures of embryo culture in IVF or intracytoplasmic sperm injection (ICSI), with a minimum difference in temperature between the two incubators of $\geq 0.5^{\circ}\text{C}$. The search process is shown in the PRISMA flow chart.

Data collection and analysis

Two review authors independently assessed trial eligibility and risk of bias and extracted data from the included studies; the third review author resolved any disagreements. We contacted trial authors to provide additional data. The primary review outcomes were live birth and miscarriage rate. Clinical pregnancy, ongoing pregnancy, multiple pregnancy and adverse events were secondary outcomes. All extracted data were dichotomous outcomes, and odds ratios (OR) were calculated with 95% confidence intervals (CIs) on an intention-to-treat basis. We assessed the overall quality of the evidence for the main comparisons using GRADE methods.

Main results

We included three RCTs, with a total of 563 women, that compared incubation of embryos at 37.0°C or 37.1°C with a lower incubator temperature (37.0°C versus 36.6°C , 37.1°C versus 36.0°C , 37.0° versus 36.5°C). Live birth, miscarriage, clinical pregnancy, ongoing pregnancy and multiple pregnancy were reported. After additional information from the authors, we confirmed one study as having no adverse events; the other two studies did not report adverse events. After additional information from the authors, one study confirmed having no adverse events. Two of the studies did not report adverse events, one study reported no adverse events. We did not perform a meta-analysis as there were not enough studies included per outcome. Live birth was not graded since there were no data of interest available. The evidence for the primary outcome, miscarriage, was of very low quality. The evidence for the secondary outcomes, clinical pregnancy, ongoing pregnancy and multiple pregnancy was also of very low quality. We downgraded the evidence because of high risk of bias (for performance bias) and imprecision due to limited included studies and wide CIs.

Only one study reported the primary outcome, live birth ($n = 52$). They performed randomisation at the level of oocytes and not per woman, and used a paired design whereby two embryos, one from 36.0°C and one from 37.0°C , were transferred. The data from this study were not interpretable in a meaningful way and therefore not presented. Only one study reported miscarriage. We are uncertain whether incubation at a lower temperature decreases the miscarriage (odds ratio (OR) 0.90, 95% CI 0.52 to 1.55; 1 study, $N = 412$; very low-quality evidence).

Of the two studies that reported clinical pregnancy, only one of them performed randomisation per woman. We are uncertain whether a lower temperature improves clinical pregnancy compared to 37.0°C for embryo incubation (OR 1.08, 95% CI 0.73 to 1.60; 1 study, $N = 412$; very low-quality evidence). For the outcome, ongoing pregnancy, we are uncertain if a lower temperature is better than 37.0°C (OR 1.10, 95% CI 0.75 to 1.62; 1 study, $N = 412$; very low quality-evidence). Multiple pregnancy was reported by two studies, one of which used a paired design, which made it impossible to report the data per temperature. We are uncertain if a temperature lower than 37.0°C reduces multiple pregnancy (OR 0.80, 95% CI 0.31 to 2.07; 1 study, $N = 412$; very low-quality evidence). There was insufficient evidence to make a conclusion regarding adverse events, as no studies reported data suitable for analysis.

Authors' conclusions

This review evaluated different temperatures for embryo culture during IVF. There is a lack of evidence for the majority of outcomes in this review. Based on very low-quality evidence, we are uncertain if incubating at a lower temperature than 37°C improves pregnancy outcomes. More RCTs are needed for comparing different temperatures of embryo culture which require reporting of clinical outcomes as live birth, miscarriage, clinical pregnancy and adverse events.

Plain language summary

Temperature of embryo culture for assisted reproduction

Review question

Cochrane review authors reviewed the evidence for culturing human embryos at different temperatures during assisted reproduction, to analyse which strategy leads to the highest live birth rate.

Background

'Infertility' is defined as the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse. In vitro fertilisation (IVF) is one of the assisted reproductive techniques that can help infertile couples to have a baby. During IVF, multiple oocytes (eggs) are retrieved from the ovaries, and are fertilised by culturing

them in a dish together with a sample of sperm, or by injecting a single sperm cell directly into the oocyte (intracytoplasmic sperm injection; ICSI). Before being transferred into the uterus the fertilised oocyte (known as an embryo), must be cultured in an incubator for several days for further development. During this incubation period, the embryo is usually cultured at a temperature of 37°C, to mimic human core body temperature. However, several studies have shown that the temperature inside the female reproductive tract may be lower than 37°C, suggesting that a lower incubator temperature might be more beneficial for embryo development. In this review we assessed different temperatures of embryo culture, which may lead to a higher live birth rate.

Study characteristics

We included three randomised controlled trials that compared 37.0°C or 37.1°C with a lower incubator temperature. The studies took place in the USA, Belgium and Egypt and included a total of 563 women who all underwent IVF/ICSI. One study reported the live birth, comparing incubation of embryos at 37.0°C with 36.0°C, one study reported the clinical pregnancy, comparing incubation at 37.1°C with 36.6°C, and one study reported multiple outcomes (miscarriage, clinical pregnancy, ongoing pregnancy and multiple pregnancy), comparing incubation of embryos at 37.0°C with 36.5°C. Two studies reported no study funding or competing interests, the other study reported no information about funding or competing interests. The evidence is current to March 2019.

Key results

Only one study reported the primary outcome, live birth, but due to a small sample size, randomisation on oocytes and paired design, no conclusions could be made. We are uncertain if incubating at a lower temperature than 37°C is beneficial for the following outcomes; miscarriage, clinical pregnancy, ongoing pregnancy and multiple pregnancy. Looking at clinical pregnancy, if women have a 55% chance of a clinical pregnancy with culturing embryos at 37.0°C, the clinical pregnancy rate using a lower temperature would be between 47% and 66%. Adverse events were mostly not reported; only one study reported no adverse events. Because the number of studies was limited, and each study reported different outcomes, more randomised controlled trials (RCTs) are needed in this field.

Quality of evidence

The quality of the evidence (using GRADE) was very low due to high risk of bias (performance bias) and imprecision (limited amount of studies and wide confidence intervals). Based on the limited and very low-quality included studies, there is no evidence that a lower temperature may enhance live birth rates or any of the other studied outcomes.

Background

Description of the condition

'Infertility' is defined as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse ([Zegers-Hochschild 2017](#)). One in six couples has been reported to experience a delay in becoming pregnant ([Boivin 2007](#)), and the absolute number of infertile couples has increased due to population growth and delayed childbearing ([Botting 2000](#); [Mascarenhas 2012](#); [Matthews 2009](#)). Infertility was ranked as the fifth-highest serious global disability among populations under the age of 60 ([WHO 2019](#)).

Description of the intervention

The aim of assisted reproductive technology is to enable couples with infertility to achieve a live birth ([HCCWG 2014](#); [Zegers-Hochschild 2017](#)). In vitro fertilisation (IVF) is one of the commonly used assisted reproductive technology procedures. IVF involves fertilisation outside the human body ([Zegers-Hochschild 2017](#)), and includes mostly controlled ovarian stimulation, oocyte retrieval, fertilisation, and embryo transfer ([Derks 2009](#)). Fertilisation is achieved in a culture dish when an oocyte and spermatozoa are allowed to fuse together, combining their genetic material ([Zegers-Hochschild 2017](#)). In some cases, intracytoplasmic sperm injection (ICSI) is used to achieve fertilisation, whereby a single spermatozoon is injected into the oocyte cytoplasm ([Zegers-Hochschild 2017](#)). After IVF or ICSI fertilisation, embryos are exposed to an in vitro environment for several days (1 to 6) before embryo transfer takes place ([Farquhar 2015](#)).

To achieve successful embryo development and clinical outcomes, the embryos must be cultured in the stable environment of an incubator ([Boone 2010](#); [Swain 2014](#)). Assisted reproductive technology laboratories are required to monitor equipment according to quality management systems mandated by the Food and Drug Administration (FDA) in the USA, the EU Tissues and Cells Directive in Europe and as described by the Cairo Consensus document ([Mortimer 2018](#)). Although we could not identify any specific recommendations concerning human embryo culture temperature, most laboratories aim to set the temperature of the incubator at 37°C, which is based on the human core body temperature ([ESHRE 2016](#); [Mortimer 2018](#); [Swain 2015](#)). However, several studies have shown that the temperature in the genital tract may differ from body temperature. Grinstead and colleagues observed lower temperatures of up to

2.3°C in the follicular fluid and ovarian stroma of human females nearing the time of ovulation ([Grinsted 1985](#)). The temperature within the genital tract of rabbits varies from 2.8°C lower in follicles to 1.4°C lower in ovarian stroma that surrounds the follicle, compared to core body temperature ([Grinsted 1980](#)). Regional differences have been reported within the genital tract of pigs and cows and these are greatest in the oviduct of estrous animals in the hours before ovulation ([Greve 1996](#); [Hunter 2000](#)). It has been found that in some animals the proximal part of the fallopian tube (isthmus) may be 1°C to 2°C lower than the distal part of the fallopian tube (ampulla), the latter is the fertilisation site ([Bahat 2003](#); [Hunter 1986](#)). These studies suggest that an in vitro incubation temperature of 37°C may not necessarily provide optimal culture conditions, and that lower temperatures may be more physiologically relevant.

Maintaining the embryos at a constant temperature is challenging, because the temperature the embryo experiences inside the incubator may be affected by a range of influences including media volume, incubator type, heat distribution within the incubator and the frequency of door openings ([SIRT 2008](#)). Embryos may also be removed from the temperature-controlled environment of the incubator and analysed under a microscope to assess their development and quality ([Armstrong 2018](#); [Nakahara 2010](#)). During embryo assessment outside the incubator, the embryos are exposed to room temperature ([Wang 2002](#)), even though clinical IVF laboratories take great care to reduce temperature fluctuations by minimising the time involved and using warmed microscope stages ([ESHRE 2016](#)). Even those embryos remaining inside the incubator can experience fluctuations in temperature, caused by loss of heat from the chamber and the time taken to regain the desired temperature after opening the incubator door ([Kelly 2010](#); [SIRT 2008](#)). Furthermore, temperatures inside an incubator may also differ depending on position within the incubator ([Monahan 2013](#)). Zhang and colleagues reported that reduction of observation frequency outside the incubator can enhance embryo quality and blastocyst formation rate ([Zhang 2010](#)). This evidence indirectly suggests that creating a stable microenvironment could have a positive effect on the formation of embryos. For example, recent time-lapse system methods allow for culturing and assessing embryos and reduce fluctuations in temperature by minimising disturbances to the culture system, by allowing the embryologist to observe the embryos without removing them from the incubator ([Armstrong 2018](#)).

How the intervention might work

The role of incubation temperatures on pregnancy rate and fertilisation is poorly understood. Although incubator temperature is commonly maintained at 37°C, there is some evidence that lower temperatures may be beneficial. Animal studies of bovines suggest sensitivity to temperature changes before and during fertilisation ([Lenz 1983](#)). Studies in hamsters suggest that temperatures a few degrees below core temperature may not be detrimental, as the hamster cell has the ability to slow its metabolism ([McKiernan 1990](#)). Similar observations led to the quiet embryo hypothesis which proposed that viable preimplantation embryos operate at metabolite and nutrient turnover rates that are lower than their less viable counterparts ([Leese 2002](#); [Leese 2008](#)). This has now been revisited by Leese and colleagues to clarify that embryos with very low metabolism may also be less viable, and that a 'just enough' (Goldilocks, or lagom) level of metabolism is most likely to be associated with embryo viability ([Leese 2016](#)). Therefore, the minimum temperature may not be as critical as the maximal temperature, because a reduction in temperature will only slow down enzymatic functions ([SIRT 2008](#)). On the other hand, temperature a few degrees above core temperature in cattle has been reported to be detrimental to cells, as it resulted in irreversible damage or death of the cell ([Putney 1988](#); [Zakari 1981](#)). A further animal study showed that hyperthermia during oocyte maturation or fertilisation adversely affects oocyte maturation and fertilisation rates and retards further embryonic development ([Sugiyama 2007](#)).

Looking at temperature and its role in human IVF clinical pregnancy rates, [Higdon 2008](#) demonstrated that when setting two incubators at 37°C and measuring their precise temperatures, the incubator with most measurements < 37°C had the highest pregnancy rate. Some laboratories set their incubator temperature around 36.5°C to 36.8°C in order to avoid the possibility of the internal temperature rising above 37°C ([Boone 2010](#); [Swain 2015](#)). [Sun 2004](#) reported the effect of high temperature on human oocytes, and found that spindles in human oocytes are sensitive to high temperature. Another study that compared three methods of temperature regulation reported that rigorous thermal control during ICSI, stabilised the meiotic spindle, and was associated with improved fertilisation and pregnancy rates ([Wang 2002](#)). As maintaining a temperature at 37°C during in vitro processes seems to be important for spindle integrity, it is also likely to be important for normal fertilisation and subsequent embryo development ([Wang 2001](#)). This review will consider the role of temperature only in embryo culture, and not in oocyte development or fertilisation, as it is possible that the role of temperature could be different at every stage of the process.

Why it is important to do this review

The laboratory phase is one of the most important steps in an IVF cycle, and therefore the incubator is an essential piece of equipment. The aim of the laboratory phase is to replicate the in vivo environment. Multiple studies have investigated different temperatures of embryo culture, one study was published recently ([De Munck 2019](#)). According to the European Society of Human Reproduction and Embryology (ESHRE) guideline there is no specific recommendation for temperature of embryo culture ([ESHRE 2016](#)). This review will evaluate the evidence of temperatures used for embryo culture and may help to improve laboratory protocols and

therefore the IVF outcomes.

Objectives

To assess different temperatures of embryo culture for human assisted reproduction, which may lead to higher live birth rates.

Methods

Criteria for considering studies for this review

Types of studies

We included published and unpublished randomised controlled trials (RCTs). We excluded non-randomised studies (e.g. studies with evidence of inadequate sequence generation, such as alternate days, patient numbers) as they are associated with a high risk of bias. We excluded cross-over trials, as the design is not valid in this context.

Types of participants

Couples with infertility undergoing an in vitro fertilisation (IVF) and/or intracytoplasmic sperm injection (ICSI).

Types of interventions

Studies comparing different temperatures of embryo culture in IVF and/or ICSI were eligible for inclusion. The minimum difference in temperature between incubators compared with each other had to be ≥ 0.5 degrees Celsius.

Types of outcome measures

Primary outcomes

- Live birth, defined as delivery of a live foetus after 20 completed weeks of gestational age
- Miscarriage (per woman), defined as pregnancy loss before 20 weeks,

Secondary outcomes

- Clinical pregnancy, defined as evidence of a gestational sac, confirmed with ultrasound
- Ongoing pregnancy, defined as evidence of a gestational sac with foetal heart motion at 12 weeks, confirmed with ultrasound
- Multiple pregnancy
- Any adverse event (including ectopic pregnancy, and foetal abnormalities)

Search methods for identification of studies

We searched for relevant studies without language restriction and in consultation with the Cochrane Gynaecology and Fertility group (CGF) Information Specialist.

Electronic searches

We searched the following databases on 6 March 2019: the Cochrane Gynaecology and Fertility (CGF) Group Specialised Register of Controlled Trials (PROCITE platform), the Cochrane Central Register of Studies Online (CRSO) (web platform), MEDLINE (Ovid platform), Embase (Ovid platform), PsycINFO (Ovid platform) and CINAHL (Ebsco platform). The MEDLINE search was combined with the Cochrane highly sensitive search strategy for identifying randomised trials which appears in the *Cochrane Handbook for Systematic Reviews of Interventions* (Chapter 6.4.11; [Higgins 2011](#)). The Embase, PsycINFO and CINAHL search strategies are combined with trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN):

www.sign.ac.uk/methodology/filters.html#random.

See [Appendix 1](#), [Appendix 2](#), [Appendix 3](#), [Appendix 4](#), [Appendix 5](#), [Appendix 6](#).

Other electronic sources of trials included the following.

- Trial registers for ongoing and registered trials: ClinicalTrials.gov at www.clinicaltrials.gov, and the World Health Organization International Clinical Trials Registry Platform search portal at apps.who.int/trialsearch (see [Appendix 7](#)).
- DARE (Database of Abstracts of Reviews of Effects) on the Cochrane Library at onlinelibrary.wiley.com (see [Appendix 8](#)).
- The Web of Knowledge at webofknowledge.com (see [Appendix 9](#)).
- OpenGrey at www.opengrey.eu for unpublished literature from Europe (see [Appendix 10](#)).
- LILACS database at lilacs.bvsalud.org/en (for trials from the Portuguese and Spanish speaking world; see [Appendix 11](#)).
- PubMed and Google Scholar (for recent trials not yet indexed in the major databases; see [Appendix 12](#) and [Appendix 13](#)).

Searching other resources

We handsearched reference lists of articles retrieved by the search and contacted experts in the field to obtain

additional data. We also handsearched relevant journals and conference abstracts that are not covered by the CGF register, in liaison with the Information Specialist.

Data collection and analysis

Selection of studies

After an initial screen of titles and abstracts retrieved by the search, conducted by review authors NB and AC, we retrieved the full texts of all potentially eligible studies. NB and AC independently examined these full-text articles for compliance with the inclusion criteria and selected studies eligible for inclusion in the review. We corresponded with study investigators as required, to clarify study eligibility. Disagreement as to study eligibility were resolved by discussion or by a third review author. We documented the selection process using a PRISMA flow chart ([Liberati 2009](#)).

Data extraction and management

Two review authors (NB and AC) independently extracted data from included studies using a data extraction form designed and pilot-tested by the review authors. Any disagreements were resolved by discussion or by a third review author. Data extraction included study characteristics and outcome data. Where studies had multiple publications, the review authors collated multiple reports of the same study, so that each study rather than each report was the unit of interest in the review, and such studies had a single study ID with multiple references. We corresponded with study investigators for further data on methods and/or results, as required.

Assessment of risk of bias in included studies

Two review authors (NB and AC) independently assessed the included studies for risk of bias using the Cochrane 'Risk of bias' assessment tool ([Higgins 2011](#)), examining: selection (random sequence generation and allocation concealment); performance (blinding of participants and personnel); detection (blinding of outcome assessors); attrition (incomplete outcome data); reporting (selective reporting); and other bias. Disagreements were resolved by discussion or by a third review author (CF). We described all judgements fully, and presented the conclusions in the 'Risk of bias' table, which was incorporated into the interpretation of review findings by means of sensitivity analyses.

We have taken care to search for within-trial selective reporting, such as trials failing to report obvious outcomes, or reporting them in insufficient detail to allow their inclusion. We sought published protocols and compared the outcomes between the protocol and the final published study.

Where identified studies failed to report the primary outcome of live birth, but did report interim outcomes, such as pregnancy, we undertook informal assessment as to whether the interim values (e.g. pregnancy rates) were similar to those reported in studies that also report live birth.

Measures of treatment effect

For dichotomous data (e.g. live birth rates), we used the number of events in the control and intervention groups of each study to calculate Mantel-Haenszel odds ratios (ORs). We reversed the direction of effect of individual studies, if required, to ensure consistency across trials. We presented 95% confidence intervals (CIs) for all outcomes. Where data to calculate ORs were not available, we utilised the most detailed numerical data available that may facilitate similar analyses of included studies. We compared the magnitude and direction of effect reported by studies with how they are presented in the review, taking account of legitimate differences.

Unit of analysis issues

The primary analysis was per woman randomised. We included per pregnancy data for some outcomes (e.g. miscarriage). If studies reported only 'per cycle' data, we contacted authors and requested 'per woman' data. We briefly summarised data that did not allow valid analysis (e.g. 'per cycle' data) in an additional table; we did not meta-analyse such data. We counted multiple live births (e.g. twins or triplets) as one live birth event. We did not include data from cross-over trials.

Dealing with missing data

We analysed the data on an intention-to-treat basis, as far as possible, and made attempts to obtain missing data from the original trialists. Where these were unobtainable, we undertook imputation of individual values for the primary outcomes only. Live births were assumed not to have occurred in participants without a reported outcome. For other outcomes, we analysed only the available data. Any imputation undertaken was subjected to sensitivity analysis.

Assessment of heterogeneity

We considered whether the clinical and methodological characteristics of the included studies were sufficiently similar for meta-analysis to provide a clinically meaningful summary. We assessed statistical heterogeneity by the measure of the I^2 statistic. An I^2 measurement greater than 50% was taken to indicate substantial heterogeneity ([Higgins 2003](#); [Higgins 2011](#)).

Assessment of reporting biases

In view of the difficulty of detecting and correcting for publication bias and other reporting biases, we aimed to minimise their potential impact by ensuring a comprehensive search for eligible studies and by being alert for

duplication of data. If there were 10 or more studies in an analysis, we used a funnel plot to explore the possibility of small study effects (a tendency for estimates of the intervention effect to be more beneficial in smaller studies).

Data synthesis

We combined the data using a fixed-effect model in the following comparisons.

- 37°C versus any lower temperature
- 36.5°C versus any lower temperature
- 36.0°C versus any lower temperature
- Other temperatures compared

An increase in the odds of a particular outcome, which may be beneficial (e.g. live birth) or detrimental (e.g. adverse effects), was displayed graphically in the meta-analyses to the right of the centre line, and a decrease in the odds of an outcome to the left of the centre line.

Subgroup analysis and investigation of heterogeneity

Where data were available, we conducted subgroup analyses for all outcomes to determine the separate evidence within the following subgroups.

- Day of embryo transfer.
- Number of embryos transferred: double embryo transfer compared to single embryo transfer.

If we detected substantial heterogeneity, we explored possible explanations in subgroup analyses (e.g. different populations) and/or sensitivity analyses (e.g. different risk of bias). We took any statistical heterogeneity into account when interpreting the results, especially if there was any variation in the direction of effect.

Sensitivity analysis

We conducted sensitivity analyses for the primary outcomes to determine whether the conclusions were robust to arbitrary decisions made regarding the eligibility and analysis. These analyses included consideration of whether the review conclusions would have differed if:

- eligibility were restricted to studies without high risk of bias, defined as low risk of random sequence generation and low risk of allocation concealment;
- a random-effects model had been adopted;
- alternative imputation strategies had been implemented; or
- the summary effect measure was relative risk rather than OR.

Overall quality of the body of evidence: 'Summary of findings' table

We prepared a 'Summary of findings' table using GRADEpro or Guideline Development Tool software ([GRADEpro GDT](#)). This table evaluated the overall quality of the body of evidence for the main review outcomes (live birth, miscarriage rate, clinical pregnancy, ongoing pregnancy, multiple pregnancy and adverse events) for the main comparison, 37.0°C versus lower temperature, using GRADE criteria (study limitations (i.e. risk of bias), consistency of effect, imprecision, indirectness and publication bias; [Schünemann 2013](#)). Judgements about evidence quality (high, moderate or low) were justified, documented, and incorporated into reporting of results for each outcome.

Results

Description of studies

See [Characteristics of included studies](#); [Characteristics of excluded studies](#); [Characteristics of ongoing studies](#).

Results of the search

We conducted the search on 6 March 2019 and retrieved 1321 studies (by using each database as stated in [Appendix 1](#), [Appendix 2](#), [Appendix 3](#), [Appendix 4](#), [Appendix 6](#), [Appendix 5](#), [Appendix 7](#), [Appendix 8](#), [Appendix 9](#), [Appendix 10](#), [Appendix 11](#), [Appendix 12](#) and [Appendix 13](#)). We found two studies by handsearching ([Massip 1986](#); [Tretiakov 1964](#)). We removed 423 duplicates, resulting in 898 studies for screening. After screening for title and abstract, 27 studies were potentially eligible and were retrieved in full text. We excluded 11 studies (see 'Excluded studies'). There was one ongoing study ([NCT03548532](#)). Fifteen studies met our inclusion criteria. Five of them were substudies; [Fawzy 2018](#) had one substudy (one conference abstract), [Hong 2014](#) had two substudies (one conference abstract, one abstract) and [De Munck 2019](#) also had two substudies (both conference abstracts). Five studies were duplicates of the included studies. Two studies were clinical trials of published studies. Therefore we ended up with three studies ([De Munck 2019](#); [Fawzy 2018](#); [Hong 2014](#)). We contacted authors to provide additional data. See PRISMA flow diagram ([Figure 1](#)).

Included studies

Study design and setting

We included three randomised controlled trials (RCTs), all single-centre studies. One study was conducted in Belgium ([De Munck 2019](#)), one in Egypt ([Fawzy 2018](#)), and one in the USA ([Hong 2014](#)).

Participants

The studies included a total of 563 infertile couples undergoing assisted reproductive technology. All articles provided characteristics of the participants. [Fawzy 2018](#) included 412 women aged up to 33 years, while [De Munck 2019](#) and [Hong 2014](#) included women aged up to 40 and 42 years (respectively), at the time of patients' in vitro fertilisation/intracytoplasmic sperm injection (IVF/ICSI) cycle. The mean age was therefore 28.53 to 28.77 years in [Fawzy 2018](#), 31.2 years in [De Munck 2019](#) and 34.2 years in [Hong 2014](#). All 412 women in [Fawzy 2018](#) underwent a fresh IVF/ICSI cycle with the first attempt (or with a previous successful attempt). Women with previous failed ICSI cycles or prior difficult embryo transfer were excluded. One or two embryos were transferred on day five. Single embryo transfer took place if women had a small uterine cavity or a previous preterm birth ([Fawzy 2018](#)). Because Hong and colleagues used a paired design, each woman that underwent IVF/ICSI had her embryos randomised to the intervention and the control group. They had no more than one prior failed IVF cycle. Fresh or frozen embryos were transferred at day six of embryo development, all originated from the same oocyte pickup. The cycles where women had a frozen embryo transfer were because they did not receive a fresh embryo transfer for prevention of delayed hyperstimulation or for embryonic/endometrial dyssynchrony. Furthermore they included participants < 35 years old who were deemed not to be ideal elective single embryo transfer candidates by their treating physician due to history of a prior failed IVF cycle ([Hong 2014](#)). De Munck and colleagues included 99 women who had fresh and/or frozen cycles, and performed single and double embryo transfer ([De Munck 2019](#)). None of the three studies provided any information about the indication for IVF/ICSI.

Interventions

[Fawzy 2018](#) compared 36.5°C with 37.0°C, [Hong 2014](#) compared 36.0°C with 37.0°C and [De Munck 2019](#) compared 36.6°C with 37.1 °C. Hong and colleagues performed a paired design whereby the women's embryos were cultured in both temperatures, with genetic testing of all embryos, and a double embryo transfer took place with one embryo from each arm. DNA fingerprinting of buccal DNA from the baby was used to identify which embryo had implanted. Information on the incubators was given for each study. Hong and colleagues used tri-gas incubators (Panasonic model MCO-5M-PA). Fawzy and colleagues used a stringent culture protocol within a single incubator (Minc-1000; Cook), using the same pH, O₂ level, culture media and volume, and dishes, from day 0 to day five or six, for both arms of the study. De Munck and colleagues used a single G210 incubator of which the calibration function was used to set temperatures of the upper row (chambers 1 to 5) as close as possible to 37.10°C (range 37.05°C to 37.15°C) and the lower row (chambers 6 to 10) to 36.60°C (range 36.59°C to 36.66°C).

Outcomes

Primary outcomes

One study reported sustained implantation rate (number of live births at ≥ 24 weeks gestation per embryo transferred). Due to paired design, as explained above, the outcome was assessed by testing buccal DNA from babies by DNA fingerprinting to identify which embryo had implanted ([Hong 2014](#)). One study reported miscarriage ([Fawzy 2018](#)).

Secondary outcomes

Two studies reported clinical pregnancy ([De Munck 2019](#); [Fawzy 2018](#)). One study reported ongoing pregnancy ([Fawzy 2018](#)). Multiple pregnancy was reported by two studies ([Fawzy 2018](#); [Hong 2014](#)). Adverse events were either not reported ([De Munck 2019](#); [Fawzy 2018](#)), or the study reported no adverse events ([Hong 2014](#)).

Funding sources

[Fawzy 2018](#) and [De Munck 2019](#) reported no study funding or competing interests. [Hong 2014](#) reported no information about funding or competing interests. For further details see [Characteristics of included studies](#).

Excluded studies

We excluded 11 studies from the review, for the following reasons.

- One was not a RCT ([Speksnijder 2013](#)).
- Four contained a different patient population than predefined ([Kelly 2010](#); [Puelker 2010](#); [Sanchez 1991](#); [Tretiakov 1964](#)).
- Six studies did not compare the intervention of interest ([Belles 2014](#); [Boone 2001](#); [Fujiwara 2007](#); [Keskintepe 2007](#); [Stoddart 2003](#); [Wang 2002](#)).

See [Characteristics of excluded studies](#) for more details.

Risk of bias in included studies

We assessed the risk of bias for each included trial in the 'Risk of bias' table, see [Characteristics of included studies](#). We summarised our findings in the 'Risk of bias' summary (see [Figure 2](#)), and in the 'Risk of bias' graph (see [Figure 3](#)). We contacted authors of all studies for additional information on risk of bias.

Allocation (selection bias)

Sequence generation

We judged all three studies to be at low risk on sequence generation. They used a random number function ([Hong 2014](#)), a random allocation sequence generator ([Fawzy 2018](#)), and a computer-generated randomisation list ([De Munck 2019](#)).

Allocation concealment

We judged two studies to be at low risk for allocation concealment for using envelopes that were sequential, opaque and sealed ([Fawzy 2018](#); [Hong 2014](#)). We judged the other study to be unclear, no precise information was described ([De Munck 2019](#)).

Blinding (performance bias and detection bias)

Blinding of participants and personnel (performance bias)

We judged all studies to be at high risk due to no blinding of (assessing) personnel. For one study, participants and clinicians were blinded, but the embryology team always had access to the culture protocols and was not blinded. Therefore we judged [Fawzy 2018](#) to be at high risk. The other study had no blinding of participants, since they were all aware of the procedure of receiving two embryos, one transferred from each group. In this same study there were no blinded personnel, since the embryologist was not blinded. Because the embryologist who was performing the grading and selection of embryos for transfer was not blinded, we judged this study to be at high risk ([Hong 2014](#)). Participants from [De Munck 2019](#) were blinded for the intervention, but personnel were not blinded, therefore we judged this to be high risk.

Blinding of outcome assessors (detection bias)

We did not consider that blinding was likely to influence findings for the review outcome, live birth rate, pregnancy rate and miscarriage rate. Therefore, we judged studies reporting these outcomes to be at low risk ([De Munck 2019](#); [Fawzy 2018](#); [Hong 2014](#)).

Incomplete outcome data (attrition bias)

Correspondence with an author from one study confirmed that there was no loss to follow-up. Intention-to-treat analysis was performed, therefore we judged [Fawzy 2018](#) to be low risk. One study did not report numbers of participants in the methods section, but they explained loss to follow-up and did perform intention-to-treat. Besides that, the first author provided clarification of the data. We considered reasons for loss to follow-up to be unrelated to the intervention, since the intervention had not taken place at that moment, therefore we judged this study to be at low risk of attrition bias ([Hong 2014](#)). We also judged [De Munck 2019](#) to be at low risk, as all results were described adequately.

Selective reporting (reporting bias)

[Fawzy 2018](#) reported all outcomes stated in the protocol, therefore we judged it to be at low risk. [Hong 2014](#) reported the outcome, mosaicism rate, in the protocol but did not report it in the article, therefore we judged this study to be at high risk for reporting bias. No protocol had been published for [De Munck 2019](#), therefore we judged this to be unclear.

Other potential sources of bias

We judged [Fawzy 2018](#) to be low risk and [Hong 2014](#) to be unclear because there was insufficient information to assess whether an important risk of bias existed. We judged [De Munck 2019](#) to be at high risk because randomisation was performed on oocytes instead of per woman.

Effects of interventions

We compared different temperatures for embryo culture to evaluate which temperature could be beneficial and may lead to a higher live birth rate. We only found two studies for one comparison, 37.0°C versus any lower temperature and added a third study for this comparison since they compared 37.1°C versus any lower.

1. Comparison of 37°C versus any lower temperature

Primary outcomes

There were too few studies to conduct any planned sensitivity analysis or subgroup analysis for all outcomes.

Live birth per woman

[Hong 2014](#) used a paired design on randomised oocytes and 36 women underwent paired transfer. Twelve of 36 women delivered a singleton, four of them had been cultured at 36.0°C (33.3%), and eight of 12 had been cultured at 37.0°C (66.7%) with a P value of 0.38. We did not rate the quality of the evidence as there were no data of interest available. The two other studies did not report the outcome live birth ([De Munck 2019](#); [Fawzy 2018](#)).

Miscarriage per woman

We are uncertain whether embryo incubation at a temperature lower than 37°C reduces the number of miscarriages (odds ratio (OR) 0.90, 95% confidence interval (CI) 0.52 to 1.55; 1 study, N = 412; very low-quality evidence). [Fawzy 2018](#) reported 29 miscarriages of 205 women (14.1%) with embryos incubated at 36.5°C and 32

miscarriages of 207 women (15.4%) with embryos incubated at 37.0°C. Based on this evidence, women have a 16% chance of a miscarriage with culturing embryos at 37°C; the miscarriage rate using a lower temperature would be between 9% and 22%. [Hong 2014](#) did not report miscarriage because it was not possible to retrieve data as they performed a paired design and used newborn buccal DNA to confirm the embryo culture temperature for the outcome, live birth. The other study did not report on miscarriage rates ([De Munck 2019](#)).

Secondary outcomes

Clinical pregnancy

We are uncertain whether a lower temperature than 37°C for embryo incubation increases clinical pregnancy (OR 1.08, 95% CI 0.73 to 1.60; 1 study, N = 412; very low quality evidence). Clinical pregnancy was reported in two studies, although only one study performed randomisation per woman ([Fawzy 2018](#)). This study reported 117 of 205 women (57.0%) who achieved clinical pregnancy with embryos cultured at 36.5°C and 114 of 207 women (55.0%) with embryos cultured at 37.0°C. Based on this evidence, women have a 55% chance of a clinical pregnancy with culturing embryos at 37°C, the clinical pregnancy rate using a lower temperature would be between 47% and 66%. We did not add the other study from [De Munck 2019](#) to the 'Summary of findings' table due to their method of randomisation on oocytes. The participants of this study had fresh or frozen cycles, and received single or double embryo transfers. For those who received a single fresh embryo transfer, 13 of 28 women with embryos cultured at 36.6°C and 23 of 31 women with embryos cultured at 37.1°C achieved clinical pregnancy (P = 0.036). For [Hong 2014](#), it was not possible to retrieve data on clinical pregnancy due to their study design.

Ongoing pregnancy

We are uncertain if embryo incubation at a lower temperature than 37°C increases ongoing pregnancy, defined as pregnancy beyond 20 weeks gestation (OR 1.10, 95% CI 0.75 to 1.62; 1 study, N = 412; very low-quality evidence). [Fawzy 2018](#) reported 101 of 205 women (49.2%) who had an ongoing pregnancy with embryos cultured at 36.5°C and 97 of 207 women (46.8%) who had an ongoing pregnancy with embryos cultured at 37.0°C. Based on the evidence of this study, women have a 47% chance of an ongoing pregnancy, the ongoing pregnancy rate using a lower temperature would be between 40% and 59%. The two other studies did not report ongoing pregnancy ([De Munck 2019](#); [Hong 2014](#)). For [Hong 2014](#) it was not possible to retrieve data on ongoing pregnancy due to their study design.

Multiple pregnancy

For the outcome, multiple pregnancy, we are uncertain whether a temperature lower than 37°C is beneficial (OR 0.80, 95% CI 0.31 to 2.07; 1 study, N = 412; very low-quality evidence). [Fawzy 2018](#) reported on multiple pregnancy: 8 of 205 women with embryos cultured at 36.5°C retrieved a twin and 10 of 207 women retrieved a twin with embryos cultured at 37.0°C. The multiple pregnancy, in this case for twins would be 5%, by using a lower temperature the multiple pregnancy rate would be between 2% and 10%. [Hong 2014](#) also reported multiple pregnancy, but since they implanted two embryos, one from each arm, we could not report on the risk of multiple pregnancy per culture temperature.

Adverse events

We requested additional data on adverse events; [Hong 2014](#) did not have any adverse events. The other two studies did not report any adverse events, such as ectopic pregnancy and foetal abnormalities ([De Munck 2019](#); [Fawzy 2018](#)).

Discussion

Summary of main results

This review evaluated different temperatures for embryo culture during assisted reproduction. There is no evidence indicating that a temperature of 36.0°C or 36.5°C compared to 37°C for culturing embryos influences live birth and miscarriage; these outcomes were reported separately in two studies ([Fawzy 2018](#); [Hong 2014](#)). Two studies reported clinical pregnancy, but only one of them performed randomisation per woman. Based on very low-quality evidence, we are uncertain of the difference between culturing embryos at 37°C or a lower temperature ([Fawzy 2018](#)). Ongoing pregnancy and multiple pregnancy were also reported by only one study, and again based on very low-quality evidence, we are uncertain of the effect on both outcomes by comparing 37°C to any lower temperature. Adverse events were either not reported, or they reported no events when authors were contacted for additional information. Due to a limited number of randomised controlled trials (RCTs) reporting different outcomes, we were not able to perform a meta-analysis. See [Summary of findings table 1](#) for a complete overview.

Overall completeness and applicability of evidence

There were only a few studies that investigated our primary and secondary outcomes, and most outcomes were only reported by one study. Due to different study designs, we described the data and did not perform a meta-analysis. Only one study reported our primary outcome, live birth ([Hong 2014](#)). This study used a paired design with very small numbers of participants. Two embryos, each cultured at a different temperature were transferred following genetic testing of euploidy, and buccal DNA of the newborn was investigated to determine the origin of the embryo. Because of this paired design, we were unable to add the specific data to [Summary of](#)

[findings table 1](#). One study that reported clinical pregnancy ([De Munck 2019](#)), performed randomisation on oocytes, therefore we did not add this data to [Summary of findings table 1](#). Although they did find differences between the groups, this study was not powered for these findings, and the results therefore, cannot be assigned to the different temperatures. They are now recruiting patients for a new powered RCT for the outcome, clinical pregnancy, with randomisation per woman ([NCT03548532](#)).

The majority of included participants in this review originated from one study ([Fawzy 2018](#)). They performed single and double embryo transfer. For [Hong 2014](#), double embryo transfer always took place due to their paired design. Where specific data were available, distinguishing between single embryo transfer and double embryo transfer, it was described in the results section. Furthermore, looking at the intervention, the comparative temperature differed for all three studies; ranging from 36.0°C to 36.6°C.

Quality of the evidence

We rated the overall quality of evidence using GRADE methods ([Schünemann 2013](#)). Only three studies were included, which mostly did not report the same outcomes. All studies had high risk of bias on one or more domains. We did not grade two studies as no data of interest were available. In determining the overall quality of evidence, we accounted for high risk of bias (performance bias) and imprecision due to the limited number of included studies and wide confidence intervals. This led us to grade the quality of evidence as very low for all our primary and secondary outcomes. See [Summary of findings table 1](#) for the overview of the quality of evidence.

Potential biases in the review process

Potentially there were limited biases during the process. We followed Cochrane methodology and two review authors completed screening and data extraction. Although we performed a highly sensitive search strategy, we may have missed some negative studies, which could result in publication bias.

Agreements and disagreements with other studies or reviews

No other systematic reviews have been published in this field. There is one ongoing trial, with randomisation per woman, that is still recruiting participants ([NCT03548532](#)). Two previous studies reported that a lower temperature may be more beneficial, but these did not fit our inclusion criteria as they were both retrospective analyses ([Higdon 2008](#); [Speksnijder 2013](#)). Higdon and colleagues showed the highest pregnancy rate for the incubator with most temperature measurements < 37°C, although the intended temperature differences between the two incubators were minimal. A retrospective analysis from Speksnijder and colleagues reported a higher fertilisation rate for the group of embryos which underwent in vitro fertilisation (IVF) and were cultured in low temperature chambers (~36.0°C), although this effect was not found for the intracytoplasmic sperm injection (ICSI) group. They found no differences for clinical pregnancies or ongoing pregnancies. These authors stated that temperature, which is a basic parameter of embryo culture, is understudied and we agree that further research is recommended on this topic.

Authors' conclusions

Implications for practice

This review evaluated different temperatures for embryo culture during assisted reproduction. There is a lack of evidence for the majority of outcomes in this review. Based on very low-quality evidence, we are uncertain if incubating at a lower temperature than 37°C improves pregnancy outcomes. More randomised controlled trials (RCTs) are needed for comparing different temperatures of embryo culture.

Implications for research

More research is needed for comparing different temperatures of embryo culture, i.e. comparing 37°C (as this is the main temperature used in the laboratories) with a temperature of at least $\geq 1.0^\circ\text{C}$ lower. RCTs including women 18 to 43 years old are required and clinical outcomes, such as live birth, miscarriage, clinical pregnancy and adverse events should be reported. Furthermore, specific information should be collected on type of incubator. With a multicentre study, a subgroup analysis for type of incubator should be incorporated.

Randomisation of women, rather than oocytes, is recommended whenever such clinical outcomes are of interest because, otherwise, selection of embryos for transfer prevents further comparison beyond this point. By randomising at the oocyte level, an approach still frequently undertaken from a laboratory perspective, the only results to compare would be fertilisation rate and quality assessment of embryos; it would not be possible to compare pregnancy rate or live birth rate, making this model less than ideal for the primary outcomes in this review.

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Contributions of authors

NB prepared the protocol and the full review with contributions from CF, AC and DB.

Declarations of interest

Nora Baak: none known

Astrid Cantineau: none known

Cindy Farquhar: none known

Daniel Brison: none known

Differences between protocol and review

We added our secondary outcomes (clinical pregnancy, ongoing pregnancy, multiple pregnancy and adverse events) to the 'Summary of findings' table.

Published notes

Characteristics of studies

Characteristics of included studies

De Munck 2019

Methods	Study design: prospective Study grouping: parallel group
Participants	Baseline characteristics <i>Number of participants:</i> 99 <i>Number of cycles:</i> 99 Women aged between 24–40 years old Inclusion criteria <ul style="list-style-type: none"> • Participants who underwent ICSI with ejaculates (fresh or frozen) • Cycles with ≥ 6 mature oocytes available for intracytoplasmic sperm injection • Extended culture to day 5, either with fresh embryo transfer or with a freeze all strategy on day 5 Exclusion criteria: no other criteria
Interventions	Intervention characteristics 36.6°C 37.1°C (control group)
Outcomes	Primary outcome: fertilisation and embryo development (top and good quality) and utilisation rate (number of embryos transferred and cryopreserved per number of mature oocytes) up to day 5 or 6 Secondary outcome: <ul style="list-style-type: none"> • Clinical pregnancy rate (presence of at least one gestational sac at ultrasonographic visualisation; multiple gestational sacs were counted as one clinical pregnancy) Pregnancy: positive β hCG blood test after 14 days after transfer Clinical pregnancy with foetal heart beat: presence of at least one viable foetus 5–7 weeks after fresh embryo transfer
Notes	Quote: "The authors report no financial or commercial conflicts of interest."

Risk of bias table

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Mature sibling oocytes were randomly allocated to one of the two study groups. The first half of the sibling oocytes was allocated to one specific group based on a computer-generated randomisation list, whereas the second half of the sibling oocytes was allocated to the other group."
Allocation concealment (selection bias)	Unclear risk	Judgement comment: oocytes were allocated to the two groups based on a randomisation list. No precise information is described
Blinding of participants and personnel (performance bias)	High risk	Judgement comment: participants from De Munck 2019 were blinded, but personnel were not blinded
Blinding of outcome assessment (detection bias)	Low risk	Judgement comment: objective outcome
Incomplete outcome data (attrition bias)	Low risk	Judgement comment: results described adequately
Selective reporting (reporting bias)	Unclear risk	Judgement comment: no protocol has been published
Other bias	High risk	Judgement comment: randomisation performed on oocytes, not on women. No miscarriage rates or live birth rates were reported.

Fawzy 2018

Methods	Study design: randomised controlled trial Study grouping: parallel group
Participants	Baseline characteristics 36.5°C <ul style="list-style-type: none"> • <i>Number of participants:</i> 205 37.0°C (control group) <ul style="list-style-type: none"> • <i>Number of participants:</i> 207 Inclusion criteria <ul style="list-style-type: none"> • Women aged between 18–33 years • BMI $\geq 30\text{kg/m}^2$ • 12 antral follicles or more • Eight or more metaphase II (MII) oocytes collected • Undergoing their first ICSI cycle or had previous successful ICSI cycle Exclusion criteria <ul style="list-style-type: none"> • Endometriosis • Poor endometrial thickness ($< 8\text{ mm}$ on the day of HCG trigger) • Previous failed ICSI cycle or experienced a difficult embryo transfer • Frozen semen or surgically retrieved spermatozoa • Ejaculate contained less than 10×10^6 spermatozoa/mL • Less than 5% progressively motile spermatozoa • Spermatozoa had severe morphological defects (globozoospermic or pinhead samples)
Interventions	Intervention characteristics 36.5°C 37.0°C (control group)
Outcomes	Clinical outcomes <i>Primary outcome</i> <ul style="list-style-type: none"> • Clinical pregnancy (foetal heartbeat at week 4 or beyond after embryo transfer) <i>Secondary outcome</i> <ul style="list-style-type: none"> • Ongoing pregnancy (pregnancy beyond 20 weeks' gestation) • Implantation (foetal heartbeat observed on ultrasound as a function of the number of embryos transferred) • Biochemical pregnancy (positive beta-HCG at 14 days after embryo transfer) • Chemical pregnancy (any biochemical pregnancy that did not continue to clinical pregnancy) • Miscarriage (any biochemical pregnancy that did not reach the ongoing pregnancy stage) Embryological outcomes : rates of blastocyst formation, high-quality blastocysts, high-quality cleavage-stage embryos on day 3 and fertilisation
Notes	They reported no study funding or competing interests NCT01706900

Risk of bias table

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Randomisation was achieved using a random allocation sequence generator by an Excel random number table (Microsoft, Redmond, WA)."
Allocation concealment (selection bias)	Low risk	Quote: "The randomisation results were allocated in sequentially numbered, opaque, sealed envelopes, which were stored in the secretary's office until the day of HCG trigger."
Blinding of participants and personnel (performance bias)	High risk	Quote: "Participants and clinicians were blinded to the allocation to 36.5 degrees Celsius or 37.0 degrees Celsius culture temperature and remained blinded at transfer. Albeit the embryology team had always access to the culture protocols and was not blinded."
Blinding of outcome assessment (detection bias)	Low risk	Judgement comment: objective outcome
Incomplete outcome data (attrition bias)	Low risk	Judgement comment: intention-to-treat analysis was performed
Selective reporting (reporting bias)	Low risk	Judgement comment: full-text reports on outcomes stated in clinicaltrials.gov.
Other bias	Low risk	No annotations

Hong 2014

Methods	Study design: randomised controlled trial Study grouping: parallel group
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Participants	<p>Baseline Characteristics</p> <p>36.0°C</p> <ul style="list-style-type: none"> • <i>Number of oocytes:</i> 399 <p>37.0°C (control group)</p> <ul style="list-style-type: none"> • <i>Number of oocytes:</i> 406 <p>Total number of participants: 70 couples. 18 patients had fewer than eight mature oocytes isolated at the time of retrieval and were not eligible to participate. 52 infertile couples with a female partner less than 42 years old with eight or more mature oocytes retrieved. No further characteristics found on the 52 infertile couples. A total of 805 mature oocytes were cultured, 399 at 36 degrees and 306 at 37 degrees.</p> <p>Inclusion criteria</p> <p>Inclusion criteria reflected the paired design of the study.</p> <ul style="list-style-type: none"> • Age \leq 42 years old at the time of the patient's IVF cycle • No more than one prior failed fresh IVF cycle • Patient < 35 years old who was deemed not to be ideal elective single embryo transfer candidate by their treating physician due to history of a prior failed IVF cycle • A minimum of eight oocytes with nuclear maturity (MII) at the time of oocyte denudation <p>Exclusion criteria</p> <ul style="list-style-type: none"> • Severe male factor infertility requiring surgical sperm extraction • Chronic anovulation • BMI > 32 kg/m² • Abnormal uterine cavity • A prior history of poor fertilisation (< 50% of MII oocytes fertilising normally) • A prior history of poor blastulation (< 10% of zygotes blastulating) <p>"Infertile couples attempting conception through IVF at Reproductive Medicine Associates of New Jersey (RMANJ) from February 2012 to December 2012 were evaluated by physicians and the clinical research team to determine their eligibility to participate in the study. Participants were observed until delivery."</p> <p>"Patients were sought who would likely produce sufficient oocytes to have several mature oocytes in each of the study groups so that each patient might effectively serve as her own control. The paired nature of the experimental design ultimately provided quality between two groups of oocytes from a single patient in a single cycle as they experienced identical endocrine milieus during follicular stimulation. Each patient's cohort of mature oocytes was split into two dishes of equivalent number and morphology (based on the embryologist's subjective assessment of cytoplasmic granularity, polar body morphology, and appearance of the zona pellucida)."</p> <p>Pretreatment:</p> <p>Note that there were no restrictions on stimulation protocols.</p>
Interventions	<p>Intervention characteristics</p> <p>36.0°C</p> <p>37.0°C (control group)</p>

Outcomes	<p><i>Primary outcome</i></p> <ul style="list-style-type: none"> Proportion of zygotes with two pronuclei (2PN) that developed into expanded blastocysts suitable for either embryo transfer or cryopreservation <p><i>Secondary outcome</i></p> <ul style="list-style-type: none"> Rates of 2PN formation per MII oocyte Number of cells in the cleavage-stage embryo on day 3 Proportion of aneuploid embryos from each group per 2PN zygote Sustained implantation rate (number of live births at ≥ 24 weeks gestation per embryo transferred)
Notes	They reported no information about funding or competing interests. We requested further information about study design; this was provided by the authors.

Risk of bias table

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "The authors used a random number function to generate a simple 1:1 randomisation to determine which of the dishes was assigned to the study group, undergoing culture at 36 C. The other dish was cultured at 37 C."
Allocation concealment (selection bias)	Low risk	Quote: "Allocation concealment was achieved using sequentially numbered, opaque, sealed envelopes." Judgement comment: sequentially, opaque, sealed envelopes used
Blinding of participants and personnel (performance bias)	High risk	Quote: "The embryologist performing grading and selecting embryos for transfer was not blinded to the temperature of culture." "The patients and physicians were aware of the plan for two embryo transfer of one from each group." Information provided by the 1st author on 13/03/2016
Blinding of outcome assessment (detection bias)	Low risk	Judgement comment: sustained implantation rate is an objective outcome. Furthermore, one embryo from each arm was transferred. Therefore judged as low risk
Incomplete outcome data (attrition bias)	Low risk	Quote: "Seventy couples volunteered and were considered for participation in the study. Of these, 18 patients had fewer than eight mature oocytes isolated at the time of retrieval and were not eligible to participate. The remaining 52 patients were enrolled. No patients were lost to follow-up observation. All delivery outcomes were available." Quote: "An intent-to-treat analysis was performed to account for all patients, including those who did not undergo transfer." Judgement comment: although no numbers of participants reported in method section, they explained loss to follow-up and did intention-to-treat. Therefore low risk. Clarification of data provided from the first author on 13 March 2016
Selective reporting (reporting bias)	High risk	Judgement comment: Clinicaltrials NCT01506089; provided mosaicism rate as outcome data, not reported in this article. Quote: "rates of mosaicism cannot be directly determined."
Other bias	Unclear risk	Judgement comment: there is insufficient information to assess whether an important risk of bias exists

Footnotes

BMI – body mass index

HCG – human chorionic gonadotropin

ICSI – intracytoplasmic sperm injection

IVF – in vitro fertilisation

Characteristics of excluded studies*Belles 2014*

Reason for exclusion	Not comparing intervention of interest
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Boone 2001

Reason for exclusion	Not comparing intervention of interest
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Fujiwara 2007

Reason for exclusion	Not comparing intervention of interest
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Kelly 2010

Reason for exclusion	Different patient population than predefined
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Keskintepe 2007

Reason for exclusion	Not comparing intervention of interest
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Puelker 2010

Reason for exclusion	Different patient population than predefined
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Sanchez 1991

Reason for exclusion	Different patient population than predefined
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Speksnijder 2013

Reason for exclusion	Different study design, not a randomised controlled trial
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Stoddart 2003

Reason for exclusion	Not comparing intervention of interest
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Tretiakov 1964

Reason for exclusion	Different patient population than predefined
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Wang 2002

Reason for exclusion	Not comparing intervention of interest
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*Footnotes***Characteristics of studies awaiting classification***Footnotes***Characteristics of ongoing studies***NCT03548532*

Study name	Embryo culture at a constant temperature of 36.6°C or 37.1°C: A randomised controlled trial
Methods	Randomised controlled trial
Participants	<p>Universitair Ziekenhuis Brussel, Belgium</p> <p><i>Inclusion criteria</i></p> <ul style="list-style-type: none"> • Day 5 transfer • Single embryo transfer • Only ejaculated sperm (fresh or frozen, autologous or heterologous) • BMI < 35 • Age < 40 years • Cycle rank < 3 for current child • Last ultrasound: 8 follicles of at least 12 mm • At least 6 mature oocytes <p><i>Exclusion criteria</i></p> <ul style="list-style-type: none"> • IVF • IVF versus ICSI • Failed fertilisation in previous cycle • No previous cycle without embryo transfer • No use of Ca ionophores for embryo quality or fertilisation problems
Interventions	Embryo culture in G210 incubator at 36.6°C versus 37.1°C
Outcomes	<p><i>Primary:</i> clinical pregnancy</p> <p><i>Secondary:</i> fertilisation rate, embryo development/utilisation rate, live birth rate of the fresh cycle, cumulative live birth rate per cycle</p>
Starting date	1 November 2017
Contact information	Neelke de Munck, neelke.demunck@uzbrussel.be
Notes	Estimated completion date: 1 September 2019

Footnotes

BMI: body mass index

ICSI: intracytoplasmic sperm injection

IVF: in vitro fertilisation

Summary of findings tables**1 37.0°C compared to any lower temperature for assisted reproduction**

37.0°C compared to any lower temperature for assisted reproduction**Patient or population:** subfertile women undergoing IVF or IVF/ICSI**Setting:** infertility treatment at clinic**Intervention:** any lower temperature**Comparison:** 37.0°C

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	Nº of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with any lower temperature	Risk with 37.0°C				
Live birth			–	(1 study)	–	Live birth rate was not graded since there were no data of interest available
Miscarriage	Study population 155 per 1000		OR 0.90 (0.52 to 1.55)	412 (1 RCT)	⊕⊕⊕⊕ Very low a,b,c	
		141 per 1000 (87 to 221)				
Clinical pregnancy	Study population 551 per 1000		OR 1.08 (0.73 to 1.60)	412 (1 RCT)	⊕⊕⊕⊕ Very low a,b,c	One study was included in the 'Summary of findings' table (Fawzy 2018). Out of the provided data from the other study (De Munck 2019), we selected the fresh cycles with single and double transfers and used those numbers for narrative data to avoid cumulative and cross-over data. Therefore, we did not include this data in the 'Summary of findings' table.
		570 per 1000 (472 to 662)				
Ongoing pregnancy	Study population 469 per 1000		OR 1.10 (0.75 to 1.62)	412 (1 RCT)	⊕⊕⊕⊕ Very low a,b,c	
		492 per 1000 (398 to 588)				
Multiple pregnancy	Study population 48 per 1000		OR 0.80 (0.31 to 2.07)	412 (1 RCT)	⊕⊕⊕⊕ Very low a,b,c	Twin pregnancy rate reported
		39 per 1000 (15 to 95)				
Adverse events – not reported	–	–	–	–	–	Adverse events were either not reported, or they reported no adverse events

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; ICSI: intracytoplasmic sperm injection; IVF: in vitro fertilisation; OR: odds ratio; RCT: randomised controlled trial

GRADE Working Group grades of evidence

High quality: further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: we are very uncertain about the estimate.

Footnotes

^aDowngraded due to high risk of bias for performance bias.

^bDowngraded as this is based on only one study with a small sample size.

^cDowngraded due to wide confidence interval, which included meaningful benefit and harm.

Additional tables

References to studies

Included studies

De Munck 2019

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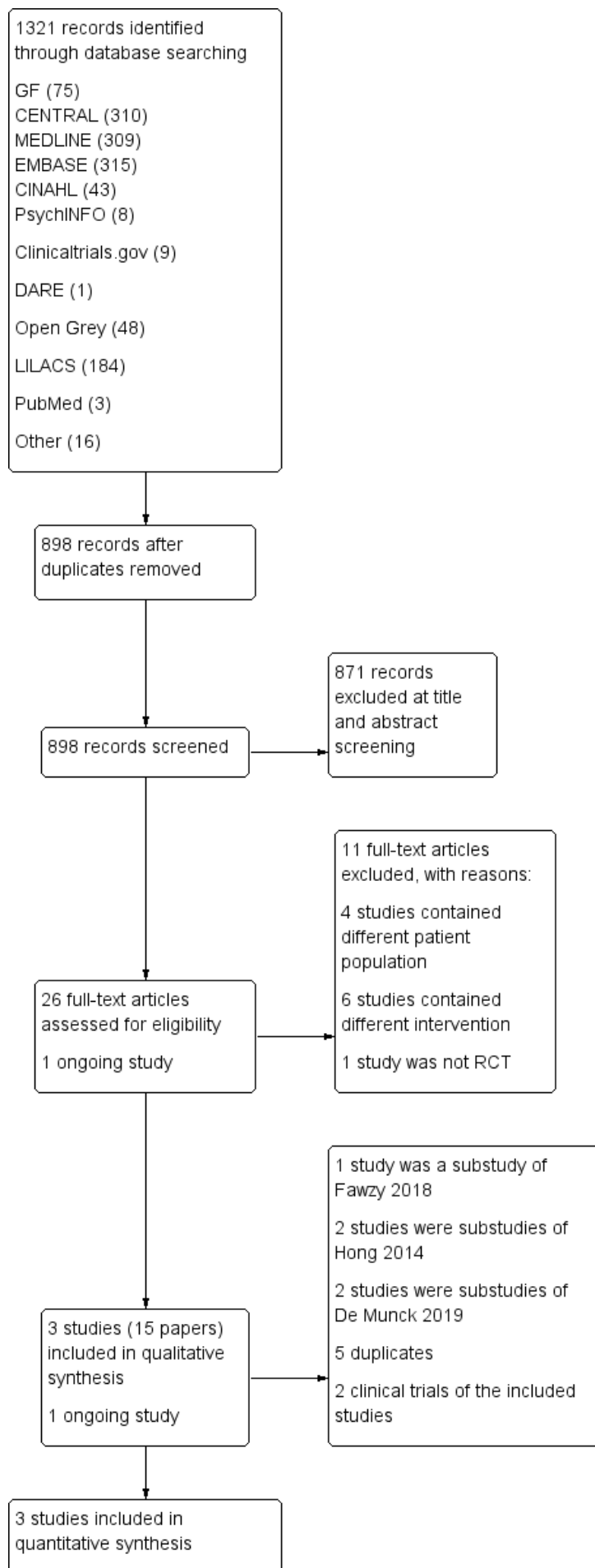
Data and analyses

1 37.0°C versus any lower temperature

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
1.1 Miscarriage rate	1	412	Odds Ratio(M-H, Fixed, 95% CI)	0.90 [0.52, 1.55]
1.2 Clinical pregnancy	1	412	Odds Ratio(M-H, Fixed, 95% CI)	1.08 [0.73, 1.60]
1.3 Ongoing pregnancy	1	412	Odds Ratio(M-H, Fixed, 95% CI)	1.10 [0.75, 1.62]
1.4 Multiple pregnancy	1	412	Odds Ratio(M-H, Fixed, 95% CI)	0.80 [0.31, 2.07]

Figures

Figure 1

**Caption**

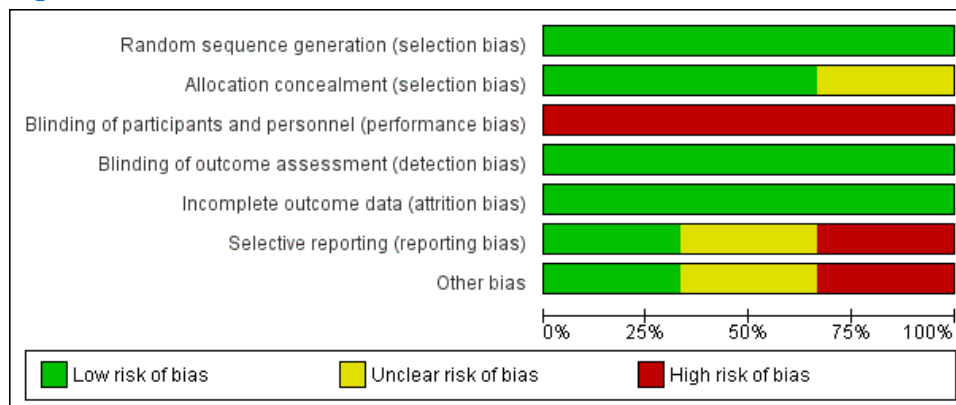
PRISMA flow diagram.

Figure 2

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
De Munck 2019	+	?	-	+	+	?	-
Fawzy 2018	+	+	-	+	+	+	+
Hong 2014	+	+	-	+	+	-	?

Caption

Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Figure 3**Caption**

Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

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Feedback

Appendices

1 Gynaecology and Fertility specialised register search strategy

Searched 06 March 2019

PROCITE platform

Keywords CONTAINS "IVF" or "in vitro fertilization" or "in-vitro fertilisation" or "ICSI" or "intracytoplasmic sperm injection" or "ET" or "Embryo" or "embryo transfer" or "blastocyst transfer" or "blastocyst" or "in-vitro fertilization" or "assisted reproduction" or "assisted reproductive technology" or "embryo coculture system" or "embryo culture" or "embryo culture media" or "embryo culture modalities" or "embryo culture techniques" or "Embryo Development" or "blastocyst culture technique" or "blastocyst development" or "blastocyst media" or "blastocyst parameters" or "blastocyst survival rate" or "blastocyst viability" or "culture techniques" or "culture incubator" or "culture" or "Culture-Media" or "media" or Title CONTAINS "IVF" or "in vitro fertilization" or "in-vitro fertilisation" or "ICSI" or "intracytoplasmic sperm injection" or "Embryo" or "in-vitro fertilization" or "ET" or "Embryo" or "embryo transfer" or "blastocyst transfer" or "blastocyst" or "in-vitro fertilization" or "assisted reproduction" or "assisted reproductive technology"

AND

Keywords CONTAINS "Temperature" or "temperature response relationship" or "humidified heated CO2" or "heated gas" or "Heat therapy" or "core temperature" or "embryo oxygen consumption" or "incubation temperature" or "incubator humidity" or "Air" or "body temperature" or "body temperature regulation" or Title CONTAINS "Temperature" or "temperature response relationship" or "humidified heated CO2" or "heated gas" or "Heat therapy" or "core temperature" or "embryo oxygen consumption" or "incubation temperature" or "incubator humidity" or "Air" or "body temperature" or "body temperature regulation" (75)

2 CENTRAL Register of Studies Online (CRSO) search strategy

Searched 06 March 2019

Web platform

- #1 MESH DESCRIPTOR Blastocyst EXPLODE ALL TREES 165
- #2 MESH DESCRIPTOR Embryo Culture Techniques EXPLODE ALL TREES 96
- #3 MESH DESCRIPTOR Fertilization in Vitro EXPLODE ALL TREES 1974
- #4 MESH DESCRIPTOR Embryo Transfer EXPLODE ALL TREES 1042
- #5 (vitro fertili?ation):TI,AB,KY 2581
- #6 icsi:TI,AB,KY 1725
- #7 (intracytoplasmic sperm injection*):TI,AB,KY 1507
- #8 blastocyst*:TI,AB,KY 964
- #9 ivf:TI,AB,KY 3861
- #10 embryo*:TI,AB,KY 5635
- #11 MESH DESCRIPTOR Embryonic Development EXPLODE ALL TREES 557
- #12 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 8293
- #13 MESH DESCRIPTOR Temperature EXPLODE ALL TREES 4229
- #14 Temperature*:TI,AB,KY 14867
- #15 microenvironment*:TI,AB,KY 618
- #16 (micro environment*):TI,AB,KY 37
- #17 thermal*:TI,AB,KY 3344
- #18 ambient:TI,AB,KY 1396
- #19 humidity:TI,AB,KY 1274
- #20 environment*:TI,AB,KY 16148
- #21 (incubat* adj2 manage*):TI,AB,KY 0
- #22 (air speed*):TI,AB,KY 5
- #23 #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 33490
- #24 #12 AND #23 239

3 MEDLINE search strategy

Searched from 1946 until 06 March 2019

OVID platform

- 1 exp Blastocyst/ (25340)
- 2 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ (39414)
- 3 vitro fertili?ation.tw. (21566)
- 4 icsi.tw. (7680)
- 5 intracytoplasmic sperm injection\$.tw. (6655)
- 6 blastocyst\$.tw. (20814)
- 7 ivf.tw. (21899)
- 8 embryo\$.tw. (333287)
- 9 exp Embryonic Development/ (55736)
- 10 exp Embryo Culture Techniques/ (3379)
- 11 or/1-10 (396762)
- 12 exp Temperature/ (399456)
- 13 temperature\$.tw. (584640)
- 14 microenvironment\$.tw. (64609)
- 15 micro-environment.tw. (1870)
- 16 thermal\$.tw. (189291)
- 17 ambient.tw. (64588)
- 18 humidity.tw. (27311)
- 19 environment\$.tw. (887634)
- 20 (incubat\$ adj2 manage\$).tw. (8)
- 21 air speed\$.tw. (187)
- 22 or/12-21 (1794769)
- 23 randomized controlled trial.pt. (476954)
- 24 controlled clinical trial.pt. (92938)
- 25 randomized.ab. (435996)
- 26 randomised.ab. (86986)
- 27 placebo.tw. (201084)
- 28 clinical trials as topic.sh. (186145)
- 29 randomly.ab. (306426)
- 30 trial.ti. (194961)
- 31 (crossover or cross-over or cross over).tw. (79363)
- 32 or/23-31 (1260991)
- 33 exp animals/ not humans.sh. (4552447)
- 34 32 not 33 (1160135)
- 35 11 and 22 and 34 (264)

4 Embase search strategy

Searched from 1980 until 06 March 2019

OID platform

- 1 exp embryo transfer/ or exp fertilization in vitro/ or exp intracytoplasmic sperm injection/ (63255)
- 2 exp embryo culture/ (7979)
- 3 (embryo\$ or blastocyst\$).tw. (369338)
- 4 (ivf or icsi).tw. (43707)
- 5 intracytoplasmic sperm injection\$.tw. (8866)
- 6 in vitro fertili?ation.tw. (28003)
- 7 or/1-6 (410412)
- 8 exp temperature/ (414655)
- 9 temperature\$.tw. (558515)
- 10 microenvironment\$.tw. (92522)
- 11 micro-environment.tw. (2915)
- 12 thermal\$.tw. (176893)
- 13 ambient.tw. (69053)
- 14 humidity.tw. (29877)
- 15 (incubat\$ adj2 manage\$).tw. (13)
- 16 air speed\$.tw. (213)
- 17 or/8-16 (929128)
- 18 7 and 17 (12011)
- 19 Clinical Trial/ (940690)
- 20 Randomized Controlled Trial/ (530583)
- 21 exp randomization/ (81186)
- 22 Single Blind Procedure/ (33789)
- 23 Double Blind Procedure/ (154472)
- 24 Crossover Procedure/ (58004)
- 25 Placebo/ (315640)
- 26 Randomi?ed controlled trial\$.tw. (195046)

27 Rct.tw. (30999)
 28 random allocation.tw. (1836)
 29 randomly.tw. (396602)
 30 randomly allocated.tw. (31528)
 31 allocated randomly.tw. (2392)
 32 (allocated adj2 random).tw. (797)
 33 Single blind\$.tw. (22020)
 34 Double blind\$.tw. (187432)
 35 ((treble or triple) adj blind\$).tw. (896)
 36 placebo\$.tw. (278166)
 37 prospective study/ (498115)
 38 or/19-37 (2194273)
 39 case study/ (59170)
 40 case report.tw. (362126)
 41 abstract report/ or letter/ (1036052)
 42 or/39-41 (1448196)
 43 38 not 42 (2144332)
 44 (exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.) (5626767)
 45 43 not 44 (1995075)
 46 18 and 45 (277)

5 PsycINFO search strategy

Searched from 1806 until 06 March 2019

OID platform

1 exp reproductive technology/ (1727)
 2 in vitro fertili?ation.tw. (709)
 3 icsi.tw. (70)
 4 intracytoplasmic sperm injection\$.tw. (54)
 5 ivf.tw. (538)
 6 (blastocyst\$ or embryo\$).tw. (10791)
 7 or/1-6 (12499)
 8 exp Temperature Effects/ (4679)
 9 temperature\$.tw. (17024)
 10 8 or 9 (18869)
 11 7 and 10 (144)
 12 random*.ti,ab,hw,id. (185938)
 13 trial*.ti,ab,hw,id. (170771)
 14 controlled stud*.ti,ab,hw,id. (11662)
 15 placebo*.ti,ab,hw,id. (38758)
 16 ((singl* or doubl* or trebl* or tripl*) and (blind* or mask*)).ti,ab,hw,id. (27758)
 17 (cross over or crossover or factorial* or latin square).ti,ab,hw,id. (28631)
 18 (assign* or allocat* or volunteer*).ti,ab,hw,id. (155595)
 19 treatment effectiveness evaluation/ (22663)
 20 mental health program evaluation/ (2058)
 21 exp experimental design/ (54771)
 22 "2000".md. (0)
 23 or/12-22 (489699)
 24 11 and 23 (7)
 25 limit 24 to yr="2018 -Current" (1)

6 CINAHL search strategy

Searched from 1961 until 06 March 2019

EBSCO platform

#	Query	Results
S21	S5 AND S8 AND S20	43
S20	S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19	1,306,088
S19	TX allocat* random*	9,893
S18	(MH "Quantitative Studies")	21,889
S17	(MH "Placebos")	11,148
S16	TX placebo*	55,507
S15	TX random* allocat*	9,893
S14	(MH "Random Assignment")	53,584
S13	TX randomi* control* trial*	164,085
S12	TX ((singl* n1 blind*) or (singl* n1 mask*)) or TX ((doubl* n1 blind*) or (doubl* n1 mask*)) or TX ((tripl* n1 blind*) or (tripl* n1 mask*)) or TX ((trebl* n1 blind*) or (trebl* n1 mask*))	1,004,440
S11	TX clinic* n1 trial*	239,105
S10	PT Clinical trial	86,754
S9	(MH "Clinical Trials+")	254,558
S8	S6 OR S7	32,112
S7	TX temperature*	28,861
S6	(MM "Temperature+")	6,846
S5	S1 OR S2 OR S3 OR S4	27,101
S4	TX embryo* or TX blastocyst*	21,984
S3	TX IVF or TX ICSI	4,562
S2	(MM "Fertilization in Vitro")	3,177
S1	TX vitro fertilisation or TX vitro fertilization	6,442

7 Clinicaltrials.gov and WHO portal for ongoing trials search strategy

Web platform

Searched 06 March 2019

embryo* and temperature* (9)

8 DARE search strategy

Web platform

Searched 06 March 2019

embryo* and temperature* (1)

9 The Web of Knowledge search strategy

Web platform

Searched 06 March 2019

embryo* and temperature*

10 OpenGrey search strategy

Web platform

Searched 06 March 2019

embryo* and temperature* (48)

11 LILACS database search strategy

Web platform

Searched 06 March 2019

embryo* and temperature* (184)

12 PubMed search strategy

Web platform

Searched 06 March 2019

(temperature* [Title] AND embryo* [Title] AND incubat* {Title/Abstract}) limited by Clinical Trials (3)

13 Google Scholar search strategy

Web platform

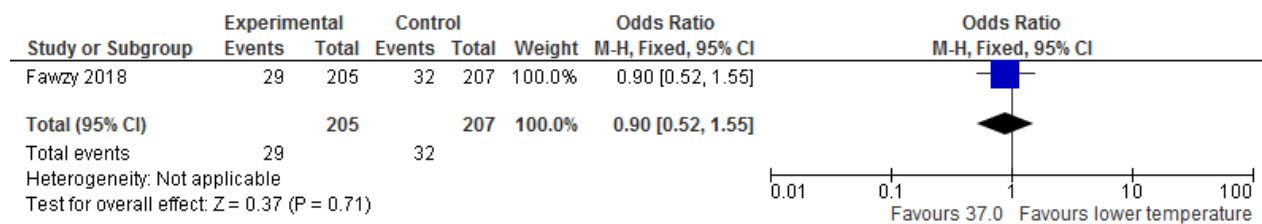
Searched 06 March 2019

(temperature* AND embryo* AND incubat*)

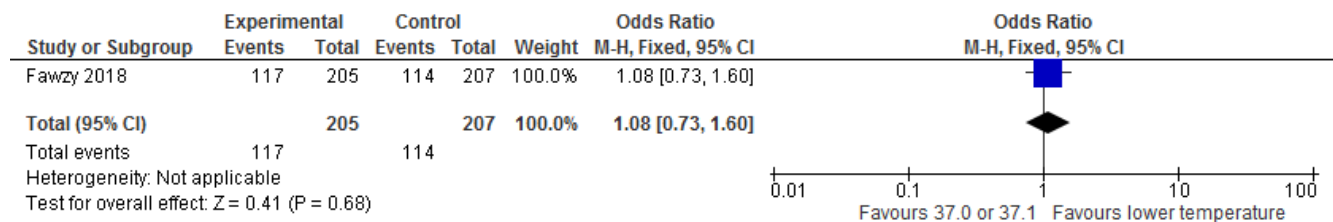
Graphs

1 – 37.0°C versus any lower temperature

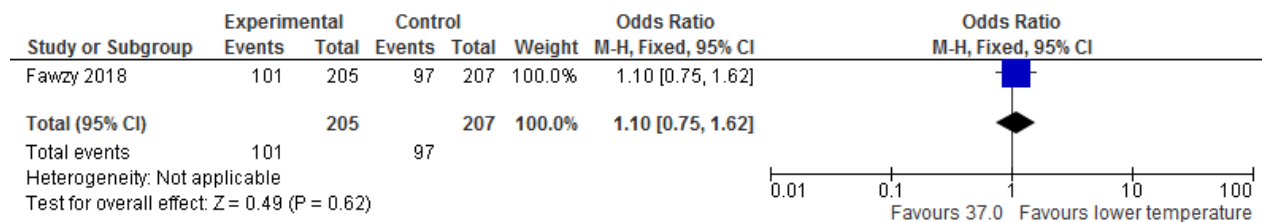
1.1 Miscarriage rate



1.2 Clinical pregnancy



1.3 Ongoing pregnancy



1.4 Multiple pregnancy

